SUPPLEMENTARY INFORMATION

MINING-DRIVEN FOREST FRAGMENTATION SHAPES MALARIA VECTOR ECOLOGY IN THE VENEZUELAN AMAZON

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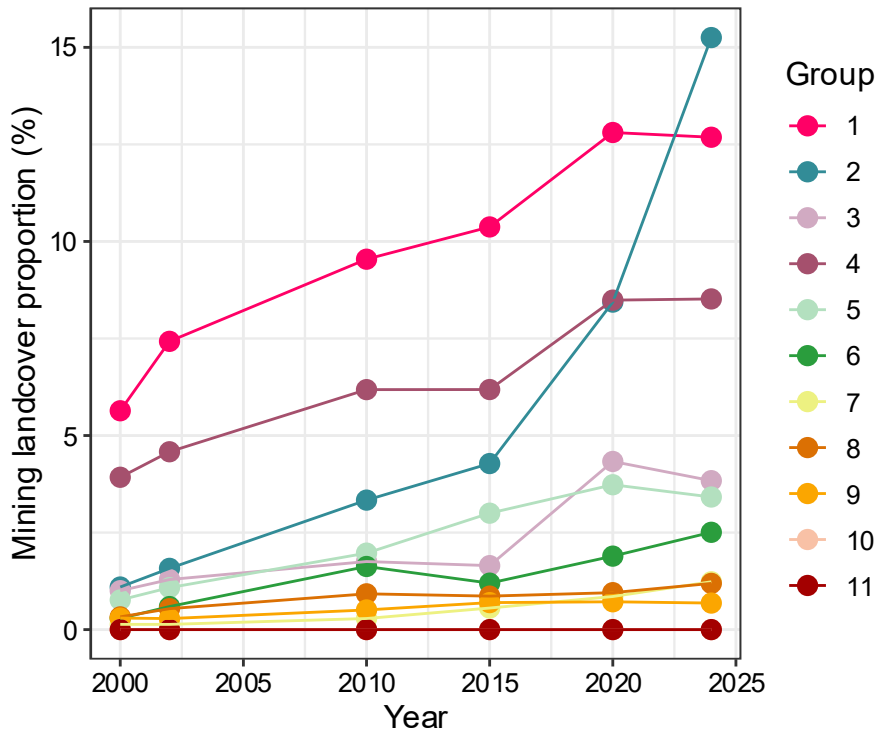
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Studied localities. Thirty-eight localities in the Sifontes municipality (Bolívar State, southern Venezuela) were included in the ecological and entomological analyses (**Supplementary Table S1**). Using raster-based analyses within 5-km buffer zones and trajectories of mining-related land cover change from 2000 to 2024, localities were classified into four groups according to the duration of mining activity and associated forest modification: no mining (Group I; 0 years), emerging mining (Group II; 5 years), intensified mining (Group III; 10 years), and established mining (Group IV; 25 years). This final categorization was informed by an initial assessment of temporal changes in mining-associated land cover, which identified distinct trajectories among 11 of the 38 localities (**Supplementary Figures S1 and S2**). The study sites ranged from permanent rural settlements (e.g., El Granzón and Tierra Blanca) to mixed rural-mining communities (San Martín de Turumbang) and forest-based mining camps (Puerto Beco, Pelota, Payapal, and Morrocoy). Population sizes varied from approximately 100 to 3,000 inhabitants, whereas mining camps were characterized by predominantly makeshift wooden housing and semi-nomadic populations.

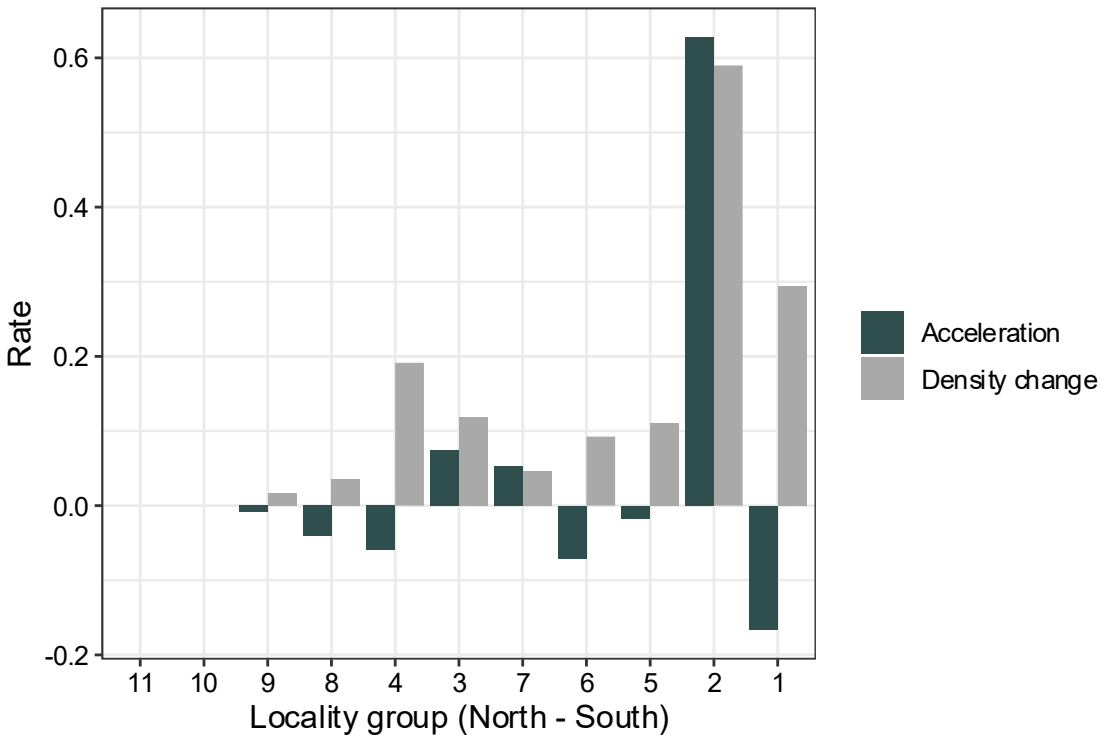
Supplementary Table S1. Study localities and mining-age (initial and final) group classification according to mining trajectory in Sifontes Municipality (Bolívar State, southern Venezuela)

Study localities (codes)*	Initial group	Final group
WUA, PEL, SCE, MPA, PLN, MAR, MOR, PBE, GRA, TBG, ROJ, GUA, BCU, CRB, POR, VIP	1	IV
PAR, SAR, CEI, SPO, EPI, POL	2	III
LOH, PLA, ARE, SMI	3	II
PAY, FBP	4	IV
LIR, LFE	5	II
ARE2, LEO	6	II
AMB, SMT	7	II
YAG	8	II
PED	9	II
BOT	10	I
ESP	11	I

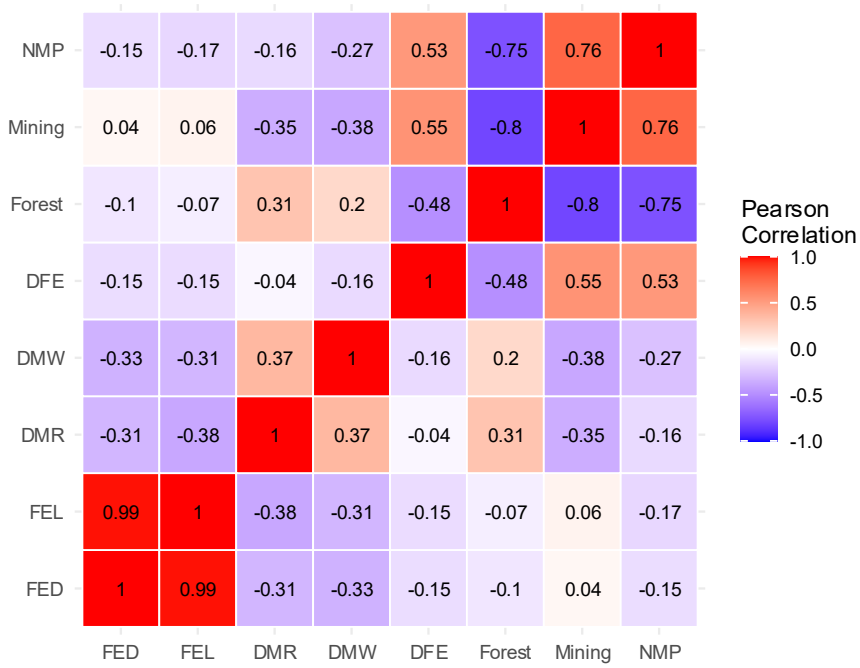
***Codes**: TBG: Tierra Blanca-El Galpon, CRB: Las Cristinas-Relave Blanco, PBE: Puerto Beco, PEL: La Pelota, SAR: San Carlos-San Antonio de Roscio, PAY: Payapal, PLA: El Placer, YAG: Yaguarin, ESP: La Esperanza, BOT: Botanamo, SMI: San Mino, GRA: El Granzon, POR: La Porfia, MOR: Morrocoy, AMB: Ambiente, SMT: San Martin de Turumbang, PED: Pedeca, ARS: Arenales-El Saco, LOH: Loma Hermosa, GUA: Guarimba, VIP: Villa Presidencial, WUA: Wuaramapate, CEI: La Ceiba, EPI: El Pinal, ROJ: Las Rojas, BCU: Brisas del Cuyuni, PAR: Paruruwaka, POL: Poleti, SPO: San Pollo, FBP: Flor del Burro-Payapal: LFE: La Fe, LIR: La Lira, ARE2: Arenales, LEO: El Leon, MPA: María Pará, PLN: La Planada, MAR: Marruecos, SCE: San Celestino.



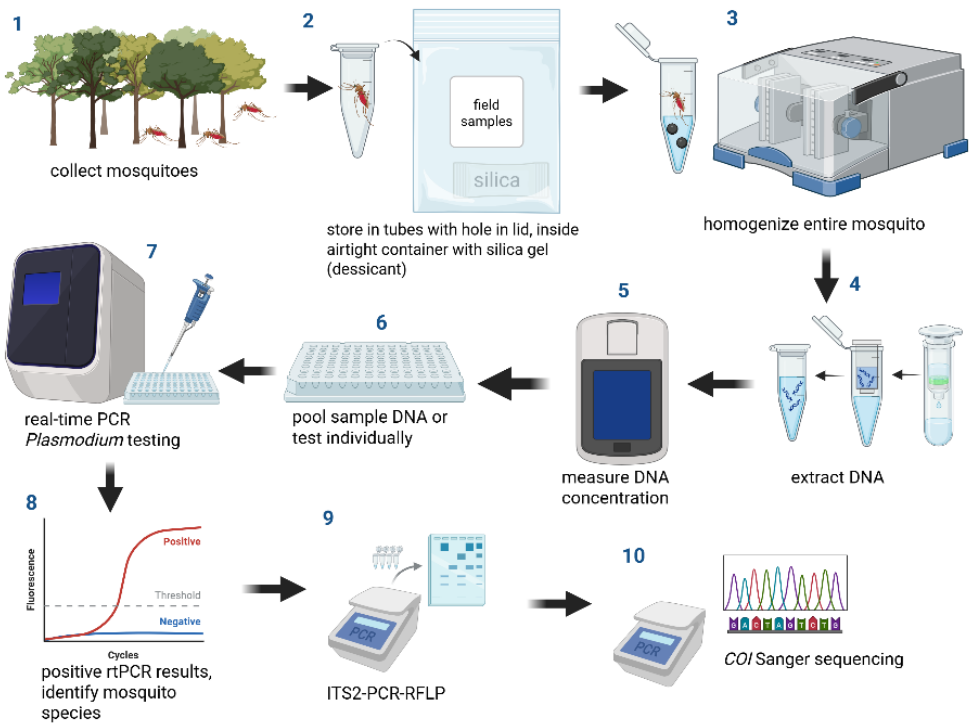
Supplementary Figure S1. Temporal changes in mining land cover (%) from 2000 to 2024 for the first group of 11 classified localities among the 38 analyzed in Sifontes Municipality, illustrating a shared mining trajectory.



Supplementary Figure S2. Estimated acceleration rates and changes in mining land-cover density between 2000 and 2024 for the first group of 11 classified localities among the 38 analyzed in Sifontes Municipality, characterized by a shared mining trajectory



Supplementary Figure S3. Pearson correlation matrix heatmap showing relationships among eight landscape metrics calculated within 2-km circular buffers around sampling localities in Sifontes Municipality. The metrics comprised mining cover, forest cover, forest edge length (FEL), forest edge density (FED), distance to mining water bodies (DMW), distance to main roads (DMR), distance to forest edges (DFE), and number of mining pits (NMP)



Supplementary Figure S4. Protocol (workflow) used for molecular identification of mosquito species (ITS2-PCR-RFLP and COI sequencing) and detection of *Plasmodium* infection (real-time PCR) in field-collected specimens in Sifontes Municipality (southern Venezuela)

Phylogenetic Analyses. Because species within the Albitarsis Complex are highly similar, Bayesian inference (BI) of COI sequences was used for species identification, providing greater reliability than BLASTn searches. Analysis of a 628-bp COI region from 238 specimens collected between 2016 and 2023, together with reference sequences and outgroups, identified Venezuelan samples as *Ny. albitarsis* F (48 unique haplotypes) and *Ny. janconnae* (13 unique haplotypes). [1–4] For the Nuneztovari Complex, COI sequences from 57 specimens were analyzed separately with publicly available sequences using a 631-bp region, identifying the collected specimens as most likely *Ny. goeldii* (8 unique haplotypes).[5–9] Analysis linked to *Ny. rondoniensis* identification is contained within Conn et al.[10]

Supplementary Table S2. Number of individual anopheline species identified molecularly by locality from 2016-2017 collections in Sifontes Municipality (Venezuela)

Locality*	*Ny. rondoniensis*	*Ny. triannulatus* s.l.	*Ny. darlingi*	*Ny. goeldii*	*Nyssorhynchus albitarsis* s.l.		Total
					Ny. albitarsis F	*Ny. janconnae*	
GRA	37	1	1	3	0	0	**42**
RAF	3	0	0	41	0	0	**44**
MES	0	0	0	0	33[&]	13	**46**
Total	**40**	**1**	**1**	**44**	**33**	**13**	**132**

Note: All samples from 2016-2017 were identified molecularly by COI sequencing as the primary purpose of these samples was to discover what species within the Nuneztovari and Albitarsis Complexes were in these regions of Venezuela

[*]GRA: Granzon, RAF: San Rafael, and MES: Mesones

[&]From all samples tested (N=132), two *Ny. albitarsis* F were found infected with *P. vivax* from Mesones (MES)

Supplementary Table S3. Number of individual anopheline species identified molecularly by locality from 2022-2023 collections in Sifontes Municipality (Venezuela). See **Supplementary Table S1** for locality code

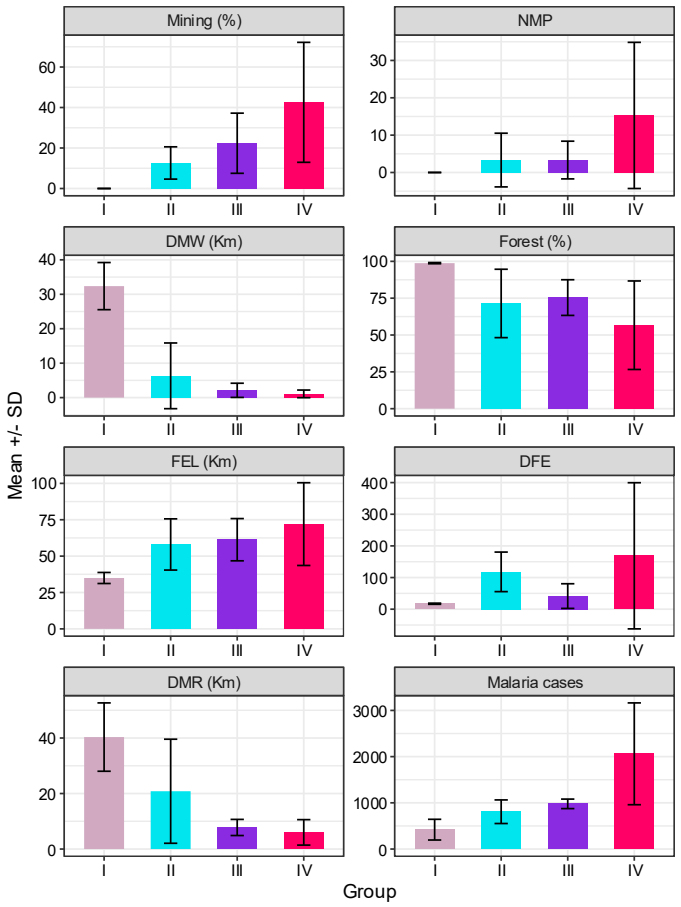
Locality	DAR	TRI	*Ny. nuneztovari* s.l.		RON	*Ny. oswaldoi* s.l.		*Ny. albitarsis* s.l.		
			NTV s.l.	GOE		OSW s.l.	OSW B	ALBT s.l.	JAN	ALBT F
TBG	28	7	0	1	3	0	0	0	14	26
CRB	0	0	0	0	0	0	0	0	9	12
PBE	29	3	0	0	5	0	0	0	0	8
PEL	2	0	8	3	7	0	0	0	15	63
SAR	0	0	0	0	0	2	0	0	0	0
PAY	0	1	9	2	10	0	0	0	1	21
PLA	0	3	0	1	0	0	1	0	0	4
YAG	0	2	4	2	0	0	3	0	0	1
ESP	1	8	0	0	0	25	0	0	0	0
BOT	0	0	0	0	0	0	0	0	0	0
SMI	0	2	0	0	0	0	0	0	0	0
GRA	185	0	12	2	12	0	0	29	0	0
POR	86	5	3	1	0	0	0	414	3	7
MOR	170	1	0	0	11	2	0	38	2	3
AMB	0	0	0	1	0	0	0	2	0	0
SMT	0	0	0	1	0	0	0	7	0	0

Species code: *Ny. darlingi* (DAR), *Ny. triannulatus* s.l. (TRI), *Ny. nuneztovari* s.l. (NTV s.l.), *Ny. goeldii* (GOE), *Ny. rondoniensis* (RON), *Ny. oswaldoi* s.l. (OSW s.l.), *Ny. oswaldoi* B (OSW B), *Ny. albitarsis* s.l. (ALBT s.l.), *Ny. janconnae* (JAN), *Ny. albitarsis* F (ALBT F)

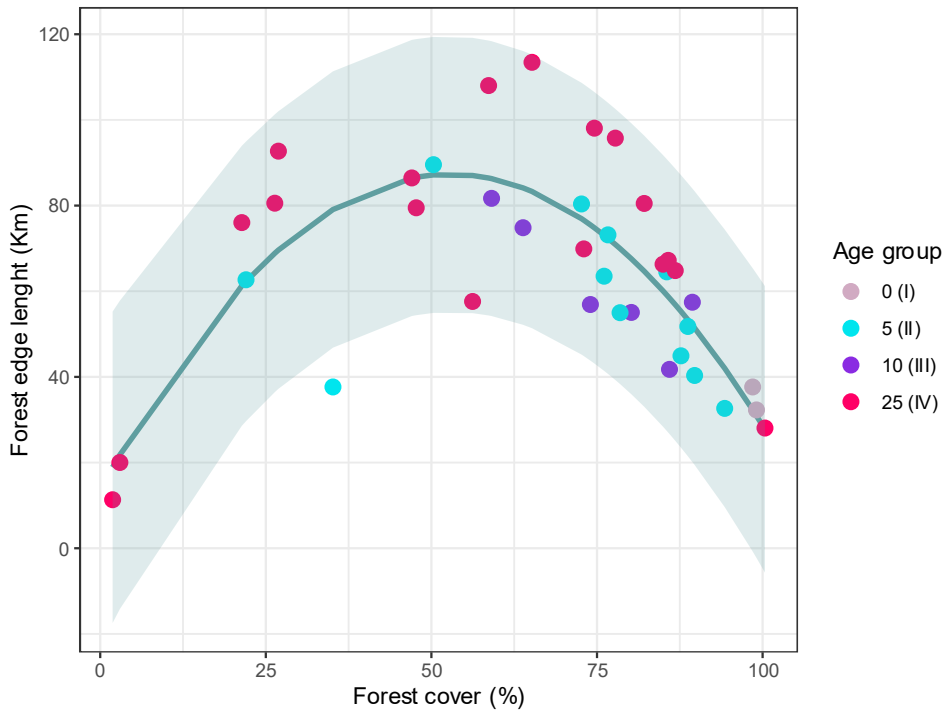
Samples collected in 2022-23 (**Supplementary Table S3)** were identified molecularly by either ITS2-PCR-RFLP or COI sequencing methods (**Supplementary Figure S4)**. All Albitarsis Complex samples that were infected with *Plasmodium* were identified using COI sequencing and phylogenetic analysis to understand which species within the complex were infected. Four vector species (*Ny. darlingi*, *Ny. albitarsis* F, *Ny. janconnae*, *Ny. rondoniensis*) were identified as infected with *Plasmodium* from four localities (**Supplementary Table S4**). Data linked to *Ny. rondoniensis* is contained within Conn et al.[10]

Supplementary Table S4. Summary of *Plasmodium* infections detected by rtPCR in samples from Sifontes Municipality, Venezuela, showing the number of infected individuals by species in positive localities.

Species	**Locality**	***P. vivax* (+)**	***P. falciparum* (+)**	***P.vivax*/*P.falciparum* (+)**
Ny. darlingi	Puerto Beco	1	-	-
	Granzón	4	-	-
	Morrocoy	24	15	2
	San Martin Turumban	1	-	-
Ny albitarsis F	Morrocoy	1	1	-
	Porfia	5	-	-
	Mesones	2		
Ny janconnae	Morrocoy		2	
	Porfia	2		



Supplementary Figure S5. Mean (± SE) values of seven landscape metrics calculated within 2-km circular buffers around sampling localities in Sifontes Municipality, grouped by locality age class (I-IV). Metrics included mining cover, forest cover, forest edge length (FEL), distance to mining water bodies (DMW), distance to main roads (DMR), distance to forest edges (DFE), and number of mining pits (NMP). The final panel shows the mean number of malaria cases for each locality group during 2022-2025



Supplementary Figure S6. Forest edge length as a function of forest cover within 2-km buffers around sampling localities in Sifontes Municipality. Localities are grouped by age class (I-IV). The fitted curve reveals a hump-shaped relationship, with edge length peaking at intermediate forest cover, suggesting that partially transformed landscapes generate more forest-edge habitat than either heavily deforested or predominantly intact forest landscapes. Shaded areas denote 95% confidence intervals

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